Chitinase and Application for Bioconversion of Chitin from Shellfish Waste

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Chitin, a linear polysaccharide of □-1,4-linked *N*-acetyl-D-glucosamine residues, is among the most abundant biopolymers on earth. Industrially, chitin has been purified from crab or shrimp waste, and further converted into valuable materials, such as chitosan, chitin-/chitosan-oligosaccharides, *N*-acetyl-D-glucosamine and glucosamine. Enzymatic conversion of chitin has been expected for providing with an alternative, environment-friendly way for its depolymerization, instead of conventional methods using chemical. In this study, we have researched about Chi40, a 40 kDa chitinase from *Streptomyces thermoviolaceus* OPC-520. The aims of our study are elucidation of the functional roles of aromatic residues within the insertion domain and at the substrate binding subsites for hydrolysis activity of Chi40 for advanced applications of the enzymes in industrial conversion.

Several conserved aromatic amino acids within insertion domain and at the substrate binding subsites were selected to replace by alanine. Site-directed mutagenesis was carried out by polymerase chain reaction using a QuickChange site-directed mutagenesis kit (Stratagene). Wild type and mutant chitinases were produced in *E. coli* cells and purified from periplasmic fraction using a procedure with 2 step affinity chromatography (Ni-agarose) and hydrophobic chromatogaraphy (Butyl Toyopearl-650). Study on the hydrolysis performance of mutant enzymes with different chitin substrates was carried out using these purified recombinant enzymes.

Our data on the activities with insoluble β-chitin and partially deacetylated water-soluble chitin of mutants suggested that only three of ten aromatic residues within insertion domain play significant roles for hydrolysis activity of Chi40. While W315 has function in preserving the structure of the enzyme, W335 and Y311 seem to have direct interaction with the substrate and important for hydrolysis activity of the enzyme with insoluble chitin.

Replacement of aromatic residues by alanine at substrate binding subsite $+1$ or $+2$ results in dramatically increasing of activity with soluble substrates. Especially, in case W149A the activity was more than 6 times in compare with wild type, which could be applied for facile production of small oligosaccharides from soluble substrates. However, hydrolysis of □- chitin was decreased in all of three W149A, W263A and F286A mutants with strongest impact have been observed in W149A. Results on hydrolysis of chitohexose in the initial stage strongly suggest the involvement of W149 in the processive activity of the enzyme. Taken together data from this study, data from three dimensional structure and data available from earlier study of ChiA (*S. marcescens*) and ChiA1 (*B. circulans*), we would propose that five studied aromatic residues W149, W263, F286, Y311 and W335 are involved in a system have function for keeping, holding and pulling the chitin chain to the active site of the enzyme. That system could be important for the enzyme to hydrolysis highly packed insoluble substrate like insoluble chitin.

This study strongly implies a large possibility of protein engineering techniques for the establishment of advanced bioconversion methods of chitinous biomass. Also, plant-originated polysaccharide biomass, composed of cellulose and xylan, is another important target for bioconversion, as rice straw, rice husk and bagasse in Vietnam. The strategies in this study should play a crucial role for development of enzymes with ideal properties for bioconversion of the biomass.